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Correlation of Oxacillin MIC with *mecA* Gene Carriage in Coagulase-Negative Staphylococci

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The National Committee for Clinical Laboratory Standards has recently changed the oxacillin breakpoint from ≥4 mg/liter to ≥0.5 mg/liter to detect methicillin-resistant coagulase-negative staphylococci (CoNS) because the previous breakpoint lacked sensitivity. To determine the correlation between the new oxacillin breakpoint and the presence of the *mecA* gene, 493 CoNS of 11 species were tested. The presence of the *mecA* gene was determined by PCR, and oxacillin susceptibility was determined by the agar dilution method with Mueller-Hinton agar containing 2% NaCl and oxacillin (0.125 to 4.0 mg/liter). The new breakpoint correctly classified all CoNS strains with *mecA* as methicillin resistant and strains of *Staphylococcus epidermidis*, *S. haemolyticus*, and *S. hominis* without *mecA* as methicillin susceptible. The breakpoint of ≥0.5 mg/liter was not specific for *S. cohnii*, *S. lugdunensis*, *S. saprophyticus*, *S. warneri*, and *S. xylosus*, in that it categorized 70 of 74 strains of these species without *mecA* (94.6%) as methicillin resistant. The results of this study indicate that the new oxacillin breakpoint accurately identifies strains of CoNS with *mecA* but is not specific for strains of certain species of CoNS without *mecA*.

Coagulase-negative staphylococci (CoNS) are a major cause of bacteremia in hospitalized patients (6, 11). Many CoNS isolates are resistant to penicillin and oxacillin by virtue of beta-lactamase and PBP 2a production, respectively (5). It is therefore common practice to use vancomycin as an initial therapy for such infections. Due to the emergence of vancomycin-resistant enterococci (17), the Hospital Infection Control Practices Advisory Committee has recommended curtailing the use of vancomycin (7). Hence, it is important for clinical laboratories to distinguish between oxacillin-susceptible and oxacillin-resistant CoNS. Recently, it was shown that the oxacillin breakpoint of ≥4 mg/liter recommended by the National Committee for Clinical Laboratory Standards (NCCLS) lacked sensitivity and was unable to classify many *mecA*-positive CoNS as oxacillin resistant. Marshall and coworkers found that lowering the oxacillin breakpoint to define resistance significantly improved the accuracy of the susceptibility tests (11). Accordingly, the NCCLS has redefined the breakpoints for oxacillin susceptibility for CoNS, so that organisms for which the MIC is ≥0.5 mg/liter are considered resistant and those for which the MIC is ≤0.25 mg/liter are considered susceptible (13). In this report, we examined the validity of these new breakpoints.

MATERIALS AND METHODS

The CoNS strains analyzed in our study were clinically significant isolates collected from patients at three teaching hospitals in our city or were obtained from other researchers. Isolates were identified by conventional biochemical tests as recommended in the *Manual of Clinical Microbiology* (9) and by other investigators (2), susceptibility to desferrioxamine (10), and cellular fatty acid profile analysis (15). The biochemical tests included acid production from carbohydrates (arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melezitose, raffinose, salicin, sorbitol, sucrose, trehalose, xylitol, and xylose); hydrolysis of arginine, esculin, and urea; decarboxylation of ornithine; detection of beta-glucosidase, coagulase, DNase, gelatinase, phosphatase, pyroglutamate aminopeptidase, oxidase, and Tween 80 lipase; acetoin pro-

duction; resistance to furazolidone and novobiocin; and nitrate reduction. Isolates were kept frozen at −70°C and subcultured twice before being tested.

The MIC of oxacillin (0.125 to 4.0 mg/liter) was determined by using agar dilution methods according to NCCLS guidelines. Briefly, for each organism, colonies isolated from overnight growth were selected to prepare direct suspensions in tryptic soy broth. The suspensions were adjusted to a 0.5 McFarland standard by using a MicroScan Turbidity Meter (Dade International Inc., Sacramento, Calif.). The 0.5 McFarland suspensions were diluted 1:10. Growth control Mueller-Hinton agar (Oxoid Inc., Nepean, Ontario, Canada) and oxacillin salt (2%) Mueller-Hinton agar plates were inoculated with the final suspensions by using a replicator delivering approximately 10^4 CFU in each spot. Plates were incubated at 35°C and were read after 24 h of incubation in ambient air. *Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 43300, and *S. aureus* ATCC 33591 were included in each run as control organisms. The MIC was recorded as the lowest concentration of oxacillin that completely inhibited growth. Oxacillin powder was obtained from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada).

To detect the *mecA* gene by PCR, growth from blood agar plates was suspended in sterile water, and the turbidity was adjusted to a 0.5 McFarland standard. To 1 ml of this suspension, an equal volume of Chelex 100 was added, followed by boiling for 7 min. After centrifugation at $18,000 \times g$ for 15 min, 1 µl of the supernatant was used in 25 µl of amplification mixture. The amplification mixture contained a standard amount of PCR buffer; 3.5 mM $MgCl_2$; 18.75 pM dUTP; 6.25 pM each dATP, dCTP, and dGTP; 5 pM each primer; and 1.25 U of *Taq* (Life Technologies, Burlington, Ontario, Canada) and 0.125 U of heat-labile uracil DNA glycosylase (Boehringer Mannheim Biochemicals). The primers used to detect the *nuc* and *mecA* genes have been previously described and amplify 310- and 270-bp fragments, respectively (3, 16). Amplification was carried out by use of a programmable GeneAmp PCR system 9600 (Perkin-Elmer Cetus) with a 10-min cycle at 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 8 min. Amplified products were detected by electrophoresis through a 2% agarose gel containing ethidium bromide (0.5 mg/liter), and bands were observed under UV light. Each run included *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, and a local epidemiological strain of methicillin-resistant *S. aureus* (MRSA) known as the Ontario strain as controls.

RESULTS

A total of 493 CoNS isolates belonging to 11 species were tested. The number of organisms of each species tested and the oxacillin MICs for strains with and without *mecA* are shown in Table 1. *mecA*-positive strains were not found among *S. capitis*, *S. lugdunensis*, *S. schleiferi*, *S. simulans*, and *S. xylosus*. Among other species, the percentage of *mecA*-positive strains varied considerably and ranged from 9.0% for *S. saprophyticus* to 83.3% for *S. haemolyticus*.

Based on the new breakpoints and the presence of the *mecA*

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TABLE 1. Correlation between the oxacillin MIC and
the presence of the *mecA* gene

Category and species	Presence (+) or absence (−) of *mecA*	Total no. of strains tested	No. of strains for which the MIC was:						
			0.125	0.25	0.5	1	2	4.0	>4.0
I									
S. epidermidis	−	101	101	0	0	0	0	0	0
	+	164	0	0	5	6	6	19	128
S. hominis	−	41	41	0	0	0	0	0	0
	+	44	0	0	2	3	3	6	30
S. haemolyticus	−	5	5	0	0	0	0	0	0
	+	25	0	0	0	0	0	0	25
II									
S. cohnii	−	10	0	0	1	0	9	0	0
	+	4	0	0	0	0	1	0	3
S. saprophyticus	−	20	0	0	4	15	1	0	0
	+	2	0	0	1	0	1	0	0
S. warneri	−	16	4	0	12	0	0	0	0
	+	4	0	0	0	0	0	0	4
III									
S. lugdunensis	−	18	0	0	7	10	1	0	0
	+	0	0	0	0	0	0	0	0
S. xylosus	−	10	0	0	4	6	0	0	0
	+	0	0	0	0	0	0	0	0
IV									
S. capitis	−	15	14	0	1	0	0	0	0
	+	0	0	0	0	0	0	0	0
S. schleiferi	−	10	10	0	0	0	0	0	0
	+	0	0	0	0	0	0	0	0
S. simulans	−	4	4	0	0	0	0	0	0
	+	0	0	0	0	0	0	0	0

gene, the organisms could be classified into the following four categories. Category I included *S. epidermidis*, *S. haemolyticus*, and *S. hominis*. More than half of the isolates in this category were *mecA* positive. The percentage of *mecA*-positive strains was highest for *S. haemolyticus* (83.3%), followed by *S. epidermidis* (61.9%) and *S. hominis* (51.8%). The MIC for all the isolates with *mecA* was ≥0.5 mg/liter, and that for isolates without *mecA* was ≤0.25 mg/liter. Category II included *S. cohnii*, *S. saprophyticus*, and *S. warneri*. These organisms had *mecA*-positive strains, but the percentage of such strains was much lower than that for the previous category, ranging from 9 to 28.5%. The oxacillin MIC for strains with *mecA* was consistently ≥0.5 mg/liter, but the MIC for 91.3% (42 of 46) of strains without *mecA* was similar. Category III included *S. lugdunensis* and *S. xylosus*. All strains of these species lacked the *mecA* gene; nevertheless, the new breakpoints classified these strains as resistant to oxacillin. Category IV included *S. capitis*, *S. schleiferi*, and *S. simulans*. These organisms were similar to those of category III in that all lacked *mecA*. However, they differed from the former in that they were correctly categorized as oxacillin sensitive by the new susceptibility breakpoints.

DISCUSSION

Detection of methicillin resistance in staphylococci is complex, mainly because the resistance is often heterogenous and only 1 of 10^4 to 10^8 cells express the resistance trait (5). Standard susceptibility testing is less accurate in determining meth-

icillin resistance in *mecA*-positive CoNS than in MRSA (18). The oxacillin MICs for *mecA*-positive CoNS have been shown to be lower than those for *mecA*-positive *S. aureus* (11), and this finding led the NCCLS to establish new oxacillin MIC breakpoints for CoNS. The correlation between the presence of the *mecA* gene and the oxacillin MIC was determined using strains of *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and less common CoNS species; however, about half of the isolates of CoNS in that study (11) were not identified to the species level. In the present study, the proportions of *mecA*-positive strains differed significantly among various species of CoNS, and the new oxacillin MIC breakpoints were less accurate when applied to *S. cohnii*, *S. saprophyticus*, *S. warneri*, *S. lugdunensis*, and *S. xylosus*. For all strains with *mecA* and 70 of 74 strains without *mecA* (94.6%) of these species, the oxacillin MICs were ≥0.5 mg/liter.

S. epidermidis, *S. haemolyticus*, and *S. hominis* are the most commonly isolated species in bacteremias due to CoNS and account for approximately 95% of such bacteremias (11, 12). The new interpretative guidelines correctly classify strains of these species with and without *mecA* as oxacillin resistant and oxacillin susceptible, respectively. Many clinical laboratories do not routinely identify the species of clinical isolates of CoNS; therefore, the oxacillin breakpoint of ≥0.5 mg/liter will correctly identify all CoNS strains with *mecA* but may also report a few strains without *mecA*, especially of species of categories II and III, as falsely methicillin resistant. However, in practice, because the vast majority of clinically significant CoNS are *S. epidermidis*, followed by *S. haemolyticus* and *S. hominis*, errors will be relatively few. Therefore, the use of the new recommendations to identify methicillin-resistant CoNS can be justified and appears to be practical.

Occasionally, CoNS other than *S. epidermidis*, *S. haemolyticus*, and *S. hominis* cause serious infections (8, 14), and in certain infections, such as shunt-associated meningitis and endocarditis, beta-lactam drugs are the preferred therapy. Susceptibility tests will define all CoNS for which the oxacillin MIC is ≥0.5 mg/liter as methicillin resistant, regardless of the species and the status of the *mecA* gene. As a result, some patients may unnecessarily be denied the benefit of relatively nontoxic and useful beta-lactam antibiotics. In order to accommodate such cases, an alternative approach to detect methicillin resistance can be adopted. Instead of relying on phenotypic susceptibility tests for the detection of methicillin resistance, tests that detect the *mecA* gene or PBP 2a can be used. PCR and DNA probes have been successfully used to detect the *mecA* gene (1, 16, 18). Archer and Pennell have demonstrated that the detection of the *mecA* gene is more sensitive and specific than oxacillin susceptibility testing in identifying methicillin-resistant CoNS (1). Recently, a latex agglutination test that detects PBP 2a has been marketed (4), although to date it has only been shown to be sensitive for MRSA. If it is demonstrated to be effective at detecting PBP 2a in CoNS, it will have several advantages. It is faster than PCR, DNA probing, and susceptibility testing, is technically simple, and can be easily performed in small laboratories. With this approach, organisms positive for the *mecA* gene or PBP 2a can be reported as methicillin resistant. Strains lacking these markers can be tested for their susceptibility to oxacillin, and breakpoints recommended for *S. aureus* can be applied, as the new guidelines for CoNS were drawn to increase the accuracy of oxacillin susceptibility tests and not for pharmacokinetic reasons. Before such recommendations are accepted, our findings must be confirmed by other studies with a larger number of CoNS isolates.

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